

Application Serial No.: 10/589,653  
Attorney Docket No.: 26068-29C

Examiner: J. Q. Ma  
Art Unit: 1797

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### LISTING OF CLAIMS

1. (Original) A microfluidic device for measuring natural motile response of a living moiety to a chemotactic agent, comprising:
  - a) a flow channel for transporting the chemotactic agent through the microfluidic device;
  - b) at least one microfluidic trench arranged beneath and substantially perpendicular to the flow channel, wherein the living moiety is introducible into the at least one microfluidic trench; and
  - c) means for measuring the response of the living moiety to the chemotactic agent;wherein the chemotactic agent is introducible into the at least one microfluidic trench to expose the living moiety to the chemotactic agent, and wherein the flow of the chemotactic agent over the at least one microfluidic trench creates a hydrodynamic stagnation of flow within the at least one microfluidic trench.
2. (Original) The microfluidic device according to claim 1, wherein the chemotactic agent is introducible into the at least one microfluidic trench by diffusion.
3. (Original) The microfluidic device according to claim 1, wherein the at least one microfluidic trench has a length dimension that is much larger than its dimensions for width and depth.
4. (Original) The microfluidic device according to claim 1, wherein the living moiety is selected from the group consisting of bacteria and cell species.
5. (Original) The microfluidic device according to claim 1, wherein the at least one microfluidic trench allows for full motility of the living moiety within the at least one microfluidic trench.

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6. (Original) The microfluidic device according to claim 1, comprising means for trapping the living moiety in the at least one microfluidic trench.
7. (Original) The microfluidic device according to claim 6, wherein the means for trapping the living moiety in the at least one microfluidic trench comprises sealing each end of the microfluidic trench.
8. (Original) The microfluidic device according to claim 7, wherein each end of the microfluidic device is sealed by means of a mechanical microvalve or an air valve.
9. (Withdrawn) The microfluidic device according to claim 1, wherein the means for trapping the living moiety in the at least one microfluidic trench comprises a roof structure over the at least one microfluidic trench.
10. (Withdrawn) The microfluidic device according to claim 9, wherein the roof structure comprises a patterned substrate surface that traps the living moiety within the at least one microfluidic trench while allowing the chemotactic agent to be introduced into the at least one microfluidic trench.
11. (Withdrawn) The microfluidic device according to claim 10, wherein the roof structure comprises a semi-permeable membrane.
12. (Withdrawn) The microfluidic device according to claim 11, wherein the semi-permeable membrane is selected from the group consisting of polycarbonate and polyethylene.
13. (Original) The microfluidic device according to claim 1, wherein the microfluidic device communicates with at least one additional microfluidic device as part of a system of devices through the control of microvalves and micropumps.

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14. (Original) The microfluidic device according to claim 1, comprising temporally varying electric fields, wherein the temporally varying electric field are applied to group, separate or select specific living moieties within the at least one microfluidic trench.
15. (Original) The microfluidic device according to claim 1, further comprising a means for creating a controlled concentration gradient of the chemotactic agent prior to introducing the chemotactic agent into the flow channel.
16. (Withdrawn) A method of using a microfluidic device to measure natural motile response of a living moiety to a chemotactic agent, the method comprising the steps of:
- a) transporting the chemotactic agent through the microfluidic device in a flow channel;
  - b) introducing at least one living moiety into at least one microfluidic trench arranged beneath and substantially perpendicular to the flow channel; and
  - c) measuring the response of the at least one living moiety to the chemotactic agent;
- wherein the chemotactic agent is introduced into the at least one microfluidic trench to expose the at least one living moiety to the chemotactic agent, and wherein the flow of the chemotactic agent over the at least one microfluidic trench creates a hydrodynamic stagnation of flow within the at least one microfluidic channel.
17. (Withdrawn) The method according to claim 16, wherein the chemotactic agent is introduced into the at least one microfluidic trench by diffusion.
18. (Withdrawn) The method according to claim 16, wherein the at least one living moiety is selected from the group consisting of bacterium and cell species.
19. (Withdrawn) The method according to claim 16, wherein the at least one microfluidic trench allows for full motility of the at least one living moiety within the at least one microfluidic trench.

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20. (Withdrawn) The method according to claim 16, wherein the at least one living moiety is trapped in the at least one microfluidic trench.
21. (Withdrawn) The method according to claim 20, wherein the at least one living moiety is trapped in the at least one microfluidic trench by sealing each end of the microfluidic trench.
22. (Withdrawn) The method according to claim 21, wherein each end of the microfluidic trench is sealed by means of a mechanical microvalve or an air valve.
23. (Withdrawn) The method according to claim 16, wherein the at least one living moiety is trapped in the at least one microfluidic trench by positioning a roof structure over the at least one microfluidic trench.
24. (Withdrawn) The method according to claim 23, wherein the roof structure comprises a patterned substrate surface that traps the at least one living moiety within the at least one microfluidic trench while allowing the chemotactic agent to be introduced into the at least one microfluidic trench.
25. (Withdrawn) The method according to claim 24, wherein the roof structure comprises a semi-permeable membrane.
26. (Withdrawn) The method according to claim 25, wherein the semi-permeable membrane is selected from the group consisting of polycarbonate and polyethylene.
27. (Withdrawn) The method according to claim 16, wherein a controlled concentration gradient of the chemotactic agent is introduced into the flow channel.
28. (Withdrawn) The method according to claim 16, wherein the at least one living

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moiety is a bacteria and the microfluidic device is used to study bacterial chemotaxis.

29. (Withdrawn) The method according to claim 16, wherein the microfluidic device is used to study cancer metastasis.

30. (Withdrawn) The method according to claim 16, where the microfluidic device is used to study stem cell populations.

31. (Withdrawn) The method according to claim 30, wherein the microfluidic device is used to study neuronal stem cells and their ability to communicate with one another in a controlled environment.

32. (Withdrawn) The method according to claim 16, wherein the microfluidic device is used to study immune response.

33. (Withdrawn) A method of fabricating a microfluidic device comprising the steps of:

- a) providing a glass slide;
- b) fabricating a layer comprising at least one microfluidic trench on the glass slide, wherein an opening of the at least one microfluidic trench is opposite the surface of the glass slide;
- c) providing a roof structure layer on the layer comprising the at least one microfluidic trench, wherein said roof structure comprises a plurality of openings; and
- d) providing a top layer comprising a flow channel, wherein said flow channel is open to the roof structure;

wherein a fluid introduced into the flow channel is introducible into the at least one microfluidic trench through the roof structure layer.

34. (Withdrawn) The method according to claim 33, wherein the top layer comprises poly-dimethylsiloxane and the flow channel comprises an inlet and an outlet.

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35. (Withdrawn) The method according to claim 34, wherein the inlet comprises means for introducing a controlled concentration gradient of the fluid into the flow channel.

36. (Withdrawn) The method according to claim 35, comprising a means for introducing a substance into the at least one microfluidic trench.

37. (Withdrawn) The method according to claim 36, further comprising sealing each end of the microfluidic trench to trap the substance within the microfluidic trench.

38. (Withdrawn) The method according to claim 33, wherein the fluid is introduced into the at least one microfluidic trench by diffusion.

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